WE CLAIM:

-1-

A modified organism for producing an antibiotic, comprising a mutation in a gene encoding an enzyme of a non-heme iron (II) dependent family of oxygenases and oxidases wherein the mutation produces the enzyme that has an amino acid residue two amino acid residues upstream of a histidine residue which is an iron ligand of the enzyme wherein the mutation renders the enzyme dependent on bicarbonate as an activator to produce the antibiotic.

-2-

A modified organism for producing penicillin, comprising a mutation in a gene encoding an isopenicillin N synthetase (IPNS) activity wherein the mutation produces the IPNS that has an amino acid residue at a position two amino acid residues upstream of a histidine residue which is an iron ligand of the IPNS wherein the mutation renders the IPNS dependent on bicarbonate as an activator to produce the penicillin.

-3-

The modified organism of Claim 2 wherein the IPNS activity comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10.

-4-

A modified organism for producing cephalosporin C, comprising a mutation in a gene encoding a deacetoxycephalosporin C synthetase (DAOCS) activity wherein the mutation produces the DAOCS that has an amino acid residue at a position two amino acid residues upstream of a histidine residue which is an iron ligand of the DAOCS wherein the mutation renders the DAOCS dependent on bicarbonate as an activator to produce the cephalosporin C.

-5-

The modified organism of Claim 4 wherein the deacetoxycephalosporin C synthetase has the amino acid set forth in SEQ ID NO:18.

-6-

A modified organism for producing cephalosporin C, comprising a mutation in a gene encoding a deacetoxycephalosporin C synthetase/deacetylcephalosporin C synthetase (DAOCS/DACS) activity wherein the mutation produces the DAOCS/DACS that has an amino acid residue at a position two amino acid residues upstream of a histidine residue which is an iron ligand of the DAOCS/DACS wherein the mutation renders the DAOCS/DACS dependent on bicarbonate as an activator to produce the cephalosporin C.

-7-

The modified organism of any one of Claims 1, 2, 4, or 6 wherein the amino acid residue is selected from the group consisting of arginine and lysine.

-8-

The modified organism of any one of Claims 1, 2, 4, or 6 wherein the organism belongs to a genus selected from the group comprising *Actinomycetes*, *Aspergillus*, *Bacillus*, *Cephalosporium*, *Cercospora*, *Escherichia*, *Eubacteria*, *Micromonospora*, *Nocardia*, *Penicillium*, *Pseudomonas*, *Streptomyces*, and filamentous fungi.

-9-

The modified organism of Claim 8 wherein the organism is a species selected from the group consisting of *Aspergillus nidulans*, *Cephalosporium acremonium*, *Penicillium chrysogenum*, *Acremonium chrysogenum*, *Emericella nidulans*, *Nocardia lactamdurans*, *Nocardia uniformus*, *Streptomyces antibioticus*, *Streptomyces anulatus*, *Streptomyces argenteolus*, *Streptomyces cattleya*, *Streptomyces chartreusis*, *Streptomyces clavuligerus*, *Streptomyces fimbriatus*, *Streptomyces flavovirens*, *Streptomyces flavus*, *Streptomyces fulvoviridis*, *Streptomyces griseus*, *Streptomyces halstedi*, *Streptomyces heteromorphus*, *Streptomyces hygroscopicus*, *Streptomyces lactamdurans*, *Streptomyces lipmanii*, *Streptomyces olivaceus*, *Streptomyces panayensis*, *Streptomyces pluracidomyceticus*, *Streptomyces rochei*, *Streptomyces sioyaensis*, *Streptomyces* sp. OA-6129, *Streptomyces* sp. KC-6643, *Streptomyces tokunomensis*, *Streptomyces viridochromogenes*, *Streptomyces wadayamensis*, *Agrobacterium*, *Gluconobacter*, *Serratia*, and *Escherichia coli*.

-10-

An isolated DNA encoding an enzyme of a non-heme iron (II) dependent family of oxygenases and oxidases useful for the synthesis of an antibiotic wherein the isolated DNA comprises a codon that has been modified to encode an amino acid which is two codons upstream of a histidine codon which is an iron ligand of the enzyme wherein the amino acid renders the enzyme dependent on bicarbonate as an activator of the enzyme.

-11-

An isolated DNA encoding an isopenicillin N synthetase (IPNS) activity wherein the isolated DNA comprises a codon that has been modified to encode an amino acid which is two codons upstream from the codon that encodes a histidine residue which is an iron ligand of the IPNS wherein the amino acid renders the IPNS dependent on bicarbonate as an activator of the IPNS.

-12-

The isolated DNA of Claim 11 wherein the IPNS activity comprises the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

-13-

An isolated DNA encoding a deacetoxycephalosporin C synthetase (DAOCS) activity wherein the isolated DNA comprises a codon that has been modified to encode an amino acid which is two codons upstream from the codon that encodes a histidine residue which is an iron ligand of the DAOCS wherein the amino acid renders the DAOCS dependent on bicarbonate as an activator of the DAOCS.

-14-

The isolated DNA of Claim 13 wherein the gene encoding deacetoxycephalosporin C synthetase has the amino acid set forth in SEQ ID NO:18.

-15-

An isolated DNA encoding a deacetoxycephalosporin C synthetase/deacetylcephalosporin C synthetase (DAOCS/DACS) activity wherein the isolated DNA comprises a codon that has been modified to encode an amino acid which is two codons upstream from the codon that encodes a histidine residue which is an iron ligand of the (DAOCS/DACS) wherein the amino acid renders the (DAOCS/DACS) dependent on bicarbonate as an activator of the (DAOCS/DACS).

-16-

The isolated DNA of any one of Claims 10, 11, 13 or 15 wherein the amino acid residue is selected from the group consisting of arginine and lysine.

-17-

The isolated DNA of any one of Claims 10, 11, 13 or 15 wherein the isolated DNA is from an organism that belongs to a genus selected from the group comprising *Actinomycetes*, *Aspergillus*, *Bacillus*, *Cephalosporium*, *Cercospora*, *Escherichia*, *Eubacteria*, *Micromonospora*, *Nocardia*, *Penicillium*, *Pseudomonas*, *Streptomyces*, and filamentous fungi.

-49-

-18-

The isolated DNA Claim 17 wherein the isolated DNA is from an organism that is a species selected from the group consisting of *Aspergillus nidulans*, *Cephalosporium acremonium*, *Penicillium chrysogenum* , *Acremonium chrysogenum*, *Emericella nidulans*, *Nocardia lactamdurans*, *Nocardia uniformus*, *Streptomyces antibioticus*, *Streptomyces anulatus*, *Streptomyces argenteolus*, *Streptomyces cattleya*, *Streptomyces chartreusis*, *Streptomyces clavuligerus*, *Streptomyces fimbriatus*, *Streptomyces flavovirens*, *Streptomyces flavus*, *Streptomyces fulvoviridis*, *Streptomyces griseus*, *Streptomyces halstedi*, *Streptomyces heteromorphus*, *Streptomyces hygroscopicus*, *Streptomyces lactamdurans*, *Streptomyces lipmanii*, *Streptomyces olivaceus*, *Streptomyces panayensis*, *Streptomyces pluracidomyceticus*, *Streptomyces rochei*, *Streptomyces sioyaensis*, *Streptomyces* sp. OA-6129, *Streptomyces* sp. KC-6643, *Streptomyces tokunomensis*, *Streptomyces viridochromogenes*, *Streptomyces wadayamensis*, *Agrobacterium*, *Gluconobacter*, and *Serratia*.

-19-

An enzyme of a non-heme iron (II) dependent family of oxygenases and oxidases which is in a pathway to produce an antibiotic, comprising a mutation which is an amino acid residue that is two amino acid residues upstream of a histidine residue which is an iron ligand of the enzyme wherein the mutation renders the enzyme dependent on bicarbonate to produce the antibiotic.

-20-

The enzyme of Claim 19 wherein the amino acid residue is selected from the group consisting of arginine and lysine.

-21-

The enzyme of Claim 19 wherein the enzyme is from an organism that belongs to a genus selected from the group comprising *Actinomycetes*, *Aspergillus*, *Bacillus*, *Cephalosporium*, *Cercospora*, *Escherichia*, *Eubacteria*, *Micromonospora*, *Nocardia*, *Penicillium*, *Pseudomonas*, *Streptomyces*, and filamentous fungi.

-22-

The enzyme Claim 21 wherein the enzyme is from an organism that is a species selected from the group consisting of *Aspergillus nidulans*, *Cephalosporium acremonium*, *Penicillium chrysogenum*, *Acremonium chrysogenum*, *Emericella nidulans*, *Nocardia lactamdurans*, *Nocardia uniformus*, *Streptomyces antibioticus*, *Streptomyces anulatus*, *Streptomyces argenteolus*, *Streptomyces cattleya*, *Streptomyces chartreusis*, *Streptomyces clavuligerus*, *Streptomyces fimbriatus*, *Streptomyces flavovirens*, *Streptomyces flavus*, *Streptomyces fulvoviridis*, *Streptomyces griseus*, *Streptomyces halstedi*, *Streptomyces heteromorphus*, *Streptomyces hygroscopicus*, *Streptomyces lactamdurans*, *Streptomyces lipmanii*, *Streptomyces olivaceus*, *Streptomyces panayensis*, *Streptomyces pluracidomyceticus*, *Streptomyces rochei*, *Streptomyces sioyaensis*, *Streptomyces* sp. OA-6129, *Streptomyces* sp. KC-6643, *Streptomyces tokunomensis*, *Streptomyces viridochromogenes*, *Streptomyces wadayamensis*, *Agrobacterium*, *Gluconobacter*, and *Serratia*.

-51-

-23-

An isopenicillin N synthetase (IPNS) to produce penicillin, comprising a mutation wherein the mutation is an amino acid residue at a position two amino acid residues upstream of a histidine residue which is an iron ligand of the IPNS wherein the mutation renders the IPNS dependent on bicarbonate as an activator to produce the penicillin.

-24-

The IPNS of Claim 23 wherein the amino acid residue is selected from the group consisting of arginine and lysine.

-25-

The IPNS of Claim 23 wherein the IPNS is from an organism that belongs to a genus selected from the group comprising *Actinomycetes*, *Aspergillus*, *Bacillus*, *Cephalosporium*, *Cercospora*, *Escherichia*, *Eubacteria*, *Micromonospora*, *Nocardia*, *Penicillium*, *Pseudomonas*, *Streptomyces*, and filamentous fungi.

-52-

-26-

The IPNS of Claim 25 wherein the IPNS is from an organism that is a species selected from the group consisting of *Aspergillus nidulans*, *Cephalosporium acremonium*, *Penicillium chrysogenum*, *Acremonium chrysogenum*, *Emericella nidulans*, *Nocardia lactamdurans*, *Nocardia uniformus*, *Streptomyces antibioticus*, *Streptomyces anulatus*, *Streptomyces argenteolus*, *Streptomyces cattleya*, *Streptomyces chartreusis*, *Streptomyces clavuligerus*, *Streptomyces fimbriatus*, *Streptomyces flavovirens*, *Streptomyces flavus*, *Streptomyces fulvoviridis*, *Streptomyces griseus*, *Streptomyces halstedi*, *Streptomyces heteromorphus*, *Streptomyces hygroscopicus*, *Streptomyces lactamdurans*, *Streptomyces lipmanii*, *Streptomyces olivaceus*, *Streptomyces panayensis*, *Streptomyces pluracidomyceticus*, *Streptomyces rochei*, *Streptomyces sioyaensis*, *Streptomyces* sp. OA-6129, *Streptomyces* sp. KC-6643, *Streptomyces tokunomensis*, *Streptomyces viridochromogenes*, *Streptomyces wadayamensis*, *Agrobacterium*, *Gluconobacter*, and *Serratia*.

-27-

The IPNS of Claim 23 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

-28-

A deacetoxycephalosporin C synthetase (DAOCS) to produce cephalosporin C, comprising a mutation wherein the mutation is an amino acid residue at a position two amino acid residues upstream of a histidine residue which is an iron ligand of the DAOCS wherein the mutation renders the DAOCS dependent on bicarbonate as an activator to produce the cephalosporin C.

-29-

The DAOCS of Claim 28 wherein the amino acid residue is selected from the group consisting of arginine and lysine.

-30-

The DAOCS of Claim 28 wherein the DAOCS is from an organism that belongs to a genus selected from the group comprising *Actinomycetes*, *Aspergillus*, *Bacillus*, *Cephalosporium*, *Cercospora*, *Escherichia*, *Eubacteria*, *Micromonospora*, *Nocardia*, *Penicillium*, *Pseudomonas*, *Streptomyces*, and filamentous fungi.

-31-

The DAOCS of Claim 30 wherein the DAOCS is from an organism that is a species selected from the group consisting of *Aspergillus nidulans*, *Cephalosporium acremonium*, *Penicillium chrysogenum*, *Acremonium chrysogenum*, *Emericella nidulans*, *Nocardia lactamdurans*, *Nocardia uniformus*, *Streptomyces antibioticus*, *Streptomyces anulatus*, *Streptomyces argenteolus*, *Streptomyces cattleya*, *Streptomyces chartreusis*, *Streptomyces clavuligerus*, *Streptomyces fimbriatus*, *Streptomyces flavovirens*, *Streptomyces flavus*, *Streptomyces fulvoviridis*, *Streptomyces griseus*, *Streptomyces halstedi*, *Streptomyces heteromorphus*, *Streptomyces hygroscopicus*, *Streptomyces lactamdurans*, *Streptomyces lipmanii*, *Streptomyces olivaceus*, *Streptomyces panayensis*, *Streptomyces pluracidomyceticus*, *Streptomyces rochei*, *Streptomyces sioyaensis*, *Streptomyces* sp. OA-6129, *Streptomyces* sp. KC-6643, *Streptomyces tokunomensis*, *Streptomyces viridochromogenes*, *Streptomyces wadayamensis*, *Agrobacterium*, *Gluconobacter*, and *Serratia*.

-32-

The deacetoxycephalosporin C synthetase of Claim 28 which has the amino acid set forth in SEQ ID NO:18.

-33-

A deacetoxycephalosporin C synthetase/deacetylcephalosporin C synthetase (DAOCS/DACS) to produce cephalosporin C, comprising a mutation wherein the mutation is an amino acid residue at a position two amino acid residues upstream of a histidine residue which is an iron ligand of the DAOCS/DACS wherein the mutation renders the DAOCS/DACS dependent on bicarbonate as an activator to produce the cephalosporin C.

-34-

The DAOCS/DACS of Claim 33 wherein the amino acid residue is selected from the group consisting of arginine and lysine.

-35-

The DAOCS/DACS of Claim 33 wherein the DAOCS/DACS is from an organism that belongs to a genus selected from the group comprising *Actinomycetes*, *Aspergillus*, *Bacillus*, *Cephalosporium*, *Cercospora*, *Escherichia*, *Eubacteria*, *Micromonospora*, *Nocardia*, *Penicillium*, *Pseudomonas*, *Streptomyces*, and filamentous fungi.

The DAOCS/DACS of Claim 35 wherein the DAOCS/DACS is from an organism that is a species selected from the group consisting of *Aspergillus nidulans*, *Cephalosporium acremonium*, *Penicillium chrysogenum*, *Acremonium chrysogenum*, *Emericella nidulans*, *Nocardia lactamdurans*, *Nocardia uniformus*, *Streptomyces antibioticus*, *Streptomyces anulatus*, *Streptomyces argenteolus*, *Streptomyces cattleya*, *Streptomyces chartreusis*, *Streptomyces clavuligerus*, *Streptomyces fimbriatus*, *Streptomyces flavovirens*, *Streptomyces flavus*, *Streptomyces fulvoviridis*, *Streptomyces griseus*, *Streptomyces halstedi*, *Streptomyces heteromorphus*, *Streptomyces hygroscopicus*, *Streptomyces lactamdurans*, *Streptomyces lipmanii*, *Streptomyces olivaceus*, *Streptomyces panayensis*, *Streptomyces pluracidomyceticus*, *Streptomyces rochei*, *Streptomyces sioyaensis*, *Streptomyces* sp. OA-6129, *Streptomyces* sp. KC-6643, *Streptomyces tokunomensis*, *Streptomyces viridochromogenes*, *Streptomyces wadayamensis*, *Agrobacterium*, *Gluconobacter*, and *Serratia*.

-37-

A method for producing an antibiotic, comprising:

(a) providing an organism comprising a mutation in an enzyme of a non-heme iron (II) dependent family of oxygenases and oxidases which is in an antibiotic synthesis pathway to produce the antibiotic wherein the mutation is an amino acid residue that is two amino acid residues upstream of a histidine residue which is an iron ligand of the enzyme wherein the mutation renders the enzyme dependent on bicarbonate as an activator;

(b) growing the organism in log phase in a culture without supplemental bicarbonate;

(c) adding the bicarbonate to activate the enzyme; and

(d) isolating the antibiotic produced by the antibiotic synthesis pathway.

-38-

A method for producing penicillin G or V, comprising:

(a) providing an organism comprising a mutation in an isopenicillin N synthetase (IPNS) wherein the mutation is an amino acid residue which is two amino acid residues upstream of a histidine residue which is an iron ligand of the IPNS wherein the mutation renders the IPNS dependent on bicarbonate as an activator;

(b) growing the organism to log phase in a culture without supplemental bicarbonate;

(c) adding the bicarbonate to activate the IPNS which converts δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine to isopenicillin N; and

(d) isolating the penicillin G or V which is produced from the isopenicillin N.

-57-

-39-

The IPNS of Claim 38 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10.

-40-

A method for producing cephalosporin C, comprising:

(a) providing an organism comprising a mutation in a deacetoxycephalosporin C synthetase (DAOCS) wherein the mutation is an amino acid residue which is two amino acid residues upstream of a histidine residue which is an iron ligand of the DAOCS wherein the mutation renders the DAOCS dependent on bicarbonate as an activator;

(b) growing the organism in log phase in a culture without supplemental bicarbonate;

(c) adding the bicarbonate to activate the DAOCS which converts isopenicillin N to cephalosporin C; and

(d) isolating the cephalosporin C which is produced from the isopenicillin N.

-41-

The method of Claim 40 wherein the gene encoding deacetoxycephalosporin C synthetase has the amino acid set forth in SEQ ID NO:18.

-42-

A method for producing cephalosporin C, comprising:

(a) providing an organism comprising a mutation in a synthetase selected from the group consisting of deacetoxycephalosporin C synthetase/deacetylcephalosporin C synthetase (DAOCS/DACS) and deacetoxycephalosporin C synthetase wherein the mutation is an amino acid residue which is two amino acid residues upstream of a histidine residue which is an iron ligand of the synthetase wherein the mutation renders the synthetase dependent on bicarbonate as an activator;

(b) growing the organism in log phase in a culture without supplemental bicarbonate;

(c) adding the bicarbonate to activate the synthetase which converts isopenicillin N to cephalosporin C; and

(d) isolating the cephalosporin C which is produced from the isopenicillin N.

-43-

The method of any one of Claims 37, 38, 40 and 42 wherein the amino acid residue is selected from the group consisting of arginine and lysine.

-44-

The method of any one of Claims 37, 38, 40 and 42 wherein the organism belongs to a genus selected from the group comprising *Actinomycetes*, *Aspergillus*, *Bacillus*, *Cephalosporium*, *Cercospora*, *Escherichia*, *Eubacteria*, *Micromonospora*, *Nocardia*, *Penicillium*, *Pseudomonas*, *Streptomyces*, and filamentous fungi.

-45-

The method of Claim 44 wherein the organism is a species selected from the group consisting of *Aspergillus nidulans*, *Cephalosporium acremonium*, *Penicillium chrysogenum*, *Acremonium chrysogenum*, *Emericella nidulans*, *Nocardia lactamdurans*, *Nocardia uniformus*, *Streptomyces antibioticus*, *Streptomyces anulatus*, *Streptomyces argenteolus*, *Streptomyces cattleya*, *Streptomyces chartreusis*, *Streptomyces clavuligerus*, *Streptomyces fimbriatus*, *Streptomyces flavovirens*, *Streptomyces flavus*, *Streptomyces fulvoviridis*, *Streptomyces griseus*, *Streptomyces halstedi*, *Streptomyces heteromorphus*, *Streptomyces hygroscopicus*, *Streptomyces lactamdurans*, *Streptomyces lipmanii*, *Streptomyces olivaceus*, *Streptomyces panayensis*, *Streptomyces pluracidomyceticus*, *Streptomyces rochei*, *Streptomyces sioyaensis*, *Streptomyces* sp. OA-6129, *Streptomyces* sp. KC-6643, *Streptomyces tokunomensis*, *Streptomyces viridochromogenes*, *Streptomyces wadayamensis*, *Agrobacterium*, *Gluconobacter*, and *Serratia*.

-46-

A method for making production of an antibiotic by an enzyme of a non-heme iron (II) dependent family of oxygenases and oxidases dependant on bicarbonate for activating the enzyme comprising mutating a codon that is two codons upstream from the codon that encodes a histidine which is an iron ligand of the enzyme in a gene that encodes the enzyme to a codon that encodes an amino acid that renders the production of the antibiotic dependent on the bicarbonate for activity.

-47-

The method of Claim 46 wherein the enzyme is selected from the group consisting of isopenicillin N synthetase (IPNS), deacetoxycephalosporin C synthetase (DAOCS), and deacetoxycephalosporin C synthetase/deacetylcephalosporin C synthetase (DAOCS/DACS).

-48-

The method of Claim 46 wherein the amino acid residue is selected from the group consisting of arginine and lysine.

-49-

The method of Claim 46 wherein the enzyme is from an organism that belongs to a genus selected from the group comprising *Actinomycetes*, *Aspergillus*, *Bacillus*, *Cephalosporium*, *Cercospora*, *Escherichia*, *Eubacteria*, *Micromonospora*, *Nocardia*, *Penicillium*, *Pseudomonas*, *Streptomyces*, and filamentous fungi.

The method of Claim 49 wherein the organism is a species selected from the group consisting of *Aspergillus nidulans*, *Cephalosporium acremonium*, *Penicillium chrysogenum*, *Acremonium chrysogenum*, *Emericella nidulans*, *Nocardia lactamdurans*, *Nocardia uniformus*, *Streptomyces antibioticus*, *Streptomyces anulatus*, *Streptomyces argenteolus*, *Streptomyces cattleya*, *Streptomyces chartreusis*, *Streptomyces clavuligerus*, *Streptomyces fimbriatus*, *Streptomyces flavovirens*, *Streptomyces flavus*, *Streptomyces fulvoviridis*, *Streptomyces griseus*, *Streptomyces halstedi*, *Streptomyces heteromorphus*, *Streptomyces hygroscopicus*, *Streptomyces lactamdurans*, *Streptomyces lipmanii*, *Streptomyces olivaceus*, *Streptomyces panayensis*, *Streptomyces pluracidomyceticus*, *Streptomyces rochei*, *Streptomyces sioyaensis*, *Streptomyces* sp. OA-6129, *Streptomyces* sp. KC-6643, *Streptomyces tokunomensis*, *Streptomyces viridochromogenes*, *Streptomyces wadayamensis*, *Agrobacterium*, *Gluconobacter*, and *Serratia*.